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Evaluation and Comparison of the Effects of Omega-3 and Zinc Supplements on Liver and Renal Parameters in Patients with Type 2 Diabetes

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Abstract

The aim of this study was to evaluate the effects of omega-3 and Zn supplements on liver markers SGPT (Serum glutamic pyruvic transaminase), SGOT(Serum glutamic oxaloacetic transaminase), ALP(Alkaline phosphatase), GGT(gamma-glutamyl transpeptidase), bilirubin and serum albumin and kidney markers (creatinine, uric acid and blood urea) in patients with type 2 diabetes. A randomized, double-blind, placebo-controlled, trial in patients with T2DM. Participants received zinc (30 mg/d) and/or omega 3 (1g/d fish oil) for 8 weeks. There was a significant decrease in SGOT blood levels in all three intervention groups, compared to the beginning of the study after 2 months (P=<0.001). Also the effect of Zn supplement was significant on the average SGOT change (P=0.013). The effect of omega-3 and Zn supplements on the average change in SGPT was significant (P <0.001). After two months of intervention, serum levels of GGT decreased significantly in Zn group and Zn with omega-3 group (P=0.045, P=0.004, respectively). No significant findings were found regarding other liver markers (P> 0.05). There was a significant reduction in the uric acid after intervention in zinc group (P >0/001). Our data indicated that Zn and omega-3 supplements alone or together can improve liver function in type 2 diabetic patients. There was no significant effect on kidney markers with omega-3 supplements.

Keywords: Type 2 Diabetes, Omega-3, Supplement, Zn, Liver; Kidney

Introduction

Diabetes is a chronic disease in which pancreatic cells lose the ability to produce insulin or the tissues become resistant to insulin (Alberti & Zimmet, 1998). According to the World Health Organization (WHO), in 2014, more than 422 million people suffered from diabetes, while the incidence of diabetes among people over the age of 18 years rose from 4.7% in 1980 to 8.5% in 2014 (WHO, 2016). The level of essential omega-3 fatty acids in various tissues is low in diabetes. So the use of these fatty acids can be effective in reducing the effects caused by deficiency such as blood pressure, hyperglycemia and hyperlipidemia (Coste et al., 2003). Zn also plays an important role in the function of over 300 enzymes in the body (Coleman, 1992). Zn urinary excretion increases in diabetes (Maret & Sandstead, 2006). On the other hand, Zn deficiency can play a role in glucose intolerance, diabetes and insulin resistance (Sun et al., 2009). Studies have shown that levels of liver enzymes such as alanine aminotransferase (ALT) and GGT increase in diabetic patients than healthy people, which is a good predictor of diabetes (Fraser et al., 2009). Omega-3 fatty acids have a positive effect on the improvement of hypertension, hyperlipidemia, endothelial dysfunction and cardiovascular disease (Kromhout et al., 2011). Studies have also indicated that these fatty acids can improve the steatosis in patients with non-alcoholic fatty liver (Parker et al., 2012). EPA (Eicosa Pentaenoic Acid) and DHA (Docosa Hexaenoic Acid) have the ability to control the metabolism of fatty acids in the liver by regulating the transcription factors (Ducheix et al., 2013; Kim et al., 2008). A study by Parker et. al. showed that the taking omega-3 fatty acids can have beneficial effects on liver markers and improve blood levels of these enzymes in hepatic patients (Parker et al., 2012). In another study, no beneficial effects were observed on the consumption of omega-3 fatty acids and the improvement of liver enzymes in diabetic patients (Dasarathy et al., 2015). It can be said that the results of the studies are still contradictory. According to the studies, Zn deficiency in patients with diabetes can be an effective factor in the development of liver and cirrhosis (Grüngreiff K, Reinhold, 2005). Zn is an essential element for the growth, evolution and normal cell differentiation which is involved in DNA synthesis, RNA

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transcription, and cell division and activation. Zn deficiency is found in many types of liver diseases, including alcoholic liver disease and viral liver disease (Mohammad et al., 2012). On the other hand, studies have shown that the taking Zn supplements can have beneficial effects on liver function and liver markers (Dashti et al., 1997). One of the most important and most dangerous complications of diabetes is diabetic nephropathy (6). Serum urea level increases in people with insulin-independent diabetes (Lehto et al., 1998). An increase in blood urea is associated with increased blood pressure, weight gain and decreased insulin sensitivity (Modan et al., 1987). In order to prevent the progression of kidney complications in diabetes, blocking the renin-angiotensin- aldosterone system (RAAS), regulating blood pressure (BP) and treating hyperglycemia is recommended (Inzucchi et al., 2012). Omega-3 fatty acids can improve kidney function by influencing kidney endothelial function, regulating blood pressure and reducing protein excretion from the kidneys (Shapiro et al., 2011). In cross-sectional studies in humans, the use of omega-3 fatty acids has been associated with decreased protein excretion from urine (Shapiro et al., 2011). A clinical trial aimed at investigating the effect of omega-3 fatty acids on kidney function of people with diabetes showed that these fatty acids can improve kidney function of these people and reduce protein excretion from urine (Han et al., 2016). In another clinical trial, no positive effect of omega-3 fatty acids on kidney function of people with diabetes was observed (Rossing et al., 1996). Oxidative stress can be one of the factors affecting the incidence of diabetic nephropathy. In diabetes, increased blood sugar can lead to the dysfunction of blood vessel walls and oxidative stress (. Kakkar et al., 1998). Zn can improve the activity of oxidative stress enzymes (Özcelik et al., 2012). Therefore, Zn supplements can improve the kidney function. Zn plays a role in the function and division of the kidney cells in the body. According to the studies, Zn deficiency causes kidney dysfunction (Tomat et al., 2011). In the body, short-chain fatty acids are converted to essential fatty acids by the desaturase enzyme. Zn acts as a cofactor in the activity of this enzyme, deficiency of the Zn resulting in a decrease in the level of omega-3 fatty acids in the serum (Maes et al., 1999). Given the deficiency of omega-3 fatty acids and Zn in the diabetic population this is the first study that uses these two supplements simultaneously to measure the effect of these two nutrients on liver and kidney markers of people with type 2 diabetes.

Materials and Methods:

This study aimed to investigate and compare the effects of omega-3 and Zn supplements on liver markers (SGPT, SGOT, ALP, GGT, bilirubin and albumin) and kidney markers (creatinine, uric acid and urea blood). Study was designed as a randomized controlled clinical trial in patients with type 2 diabetes for 2 months and was conducted in Imam Khomeini Hospital of Urmia (affiliated to the University of Medical Sciences, West Azerbaijan, Iran). Eligible volunteers who had been suffering from diabetes for at least two years, were taking metformin or glibenclamide, statin and serum lipid lowering drugs, calcium blocker and ACE-I. The volunteers aged between 18 and 65. People who were taking insulin, omega-3 or Zn supplements three months before the study, warfarin and heparin, diluted blood medicines, with omega-3 and fish allergies, weighing more than 150 Kg, pregnant and lactating were excluded from the study. Eligible people were given information about the subject of the study, the goals, details, and importance of the research, and, if willing, the individuals completed their written informed consent. They noted that if they did not want to cooperate during the project, they can be excluded from the study and any recorded information will be completely confidential. The study was registered at the Iranian Center for Clinical Trials Registration with IRCT20180201038585N1.

Intervention and Randomization:In this study, 100 patients with type 2 diabetes were randomly divided into four intervention and control groups: omega-3 (omega-3 fatty acid supplements with fish oil source, each one- gram capsule containing 180 mg of EPA and 120 mg of DHA), Zn (Zn supplement, each capsule containing 30 mg Zn gluconate), Zn and omega-3 group and control group. The capsules used in the control group were similar to the capsule of the intervention groups in terms of shape and color. Randomization was assigned through a random assignment list. The omega-3 and Zn supplements were taken with meals at noon and evening, respectively. Supplements were delivered at the beginning of each month in the form of 30 packs. in addition to the weekly phone calls, all individuals were asked to contact the Imam Khomeini Hospital on the 30th and 60th day after the intervention and bring with them the blank sheets of supplements.

Study measures:

A personal information questionnaire was used to collect demographic information. Height (to ±0.1 cm) was measured at baseline with a wall-mounted stadiometer and body weight (to ±100 g) was measured with an electronic calibrated scale. (BMI) was calculated by dividing the weight (kg) into the square of height (m^2). The MET (Metabolic Equivalent of Task) questionnaire was used to measure physical activity of the subjects.

Collection of Dietary Information: All subjects were asked not to change their diet and lifestyle during the intervention. In order to evaluate the dietary changes, 24-hour dietary recall was used at the beginning and the end of the study in 3 days of the week. Dietary information was evaluated through using N4 software version 3.5.2.

Blood Collection: Blood samples (5cc) were taken once at the beginning of the study and at the end of the study, while the patients were fasting for 10 hours. Serums were isolated after centrifugation at a rate of 3000 r/min for 15 minutes and used for biochemical tests. It should be noted that all biochemical tests were carried out at the beginning and at the end of the study by the same subjects under the supervision of the laboratory researcher. The uric acid level in the serum was evaluated by enzymatic method using Uricase-POD

enzyme. The urea level was determined by enzymatic method using urease enzyme. We used colorimetric approach to evaluate the creatinine and albumin. Liver enzymes were measured using the BT3000 auto-analyzer and Pars Company's kits.

Sample Size and Statistical Analysis:

A total of 100 type-2 diabetic patients were randomly divided into four intervention and control group. In this study, a single-sample Kolmogorov-Smirnov test was used to check the normal distribution of data. In order to identify confounding variables, the literature review method was used and the four groups with Chi-square and Kruskal-Wallis single-valued tests were compared. The variables that were less than 0.25 were selected as confounding variables. Finally, the confounding variables used in multivariate statistical models were as follows: Age, level of education, job, change in dietary energy, dietary fat change, dietary SFA changes, dietary PUFA changes, dietary MUFA changes, dietary fiber changes, and insoluble fiber changes. In addition, the ANOVA analysis model was used for multivariate modeling along with regulating the effect of variables. In all cases, the variable "the change over time" is considered. The mean, standard deviations, median, inter-quartile domain and frequency distribution tables (abundance and percentage) have been used in order to describe the quantitative data for frequency data. It should be noted that all stages of statistical analysis were performed using SPSS software version 22, under the significance level of 0.05.

Results:

Of the 100 patients who participated in the study, 83 patients completed the intervention. In Table 1, some demographic variables are compared in four experimental groups. Kruskal-Wallis test showed that there was no significant difference between the four groups in terms of age, duration of disease and BMI ($P < 0.05$). In addition, the results of Chi- square test showed that there was no significant association between sex, education level, type of occupation of patients and place of residence and type of experimental group.

Table 2 shows the dietary intake of patients with type 2 diabetes in the four groups under study. The ANOVA analysis showed that there was a statistically significant difference between the four groups in terms of receiving SFA, PUFA, MUFA, dietary fiber and insoluble fiber ($P < 0.05$).

Table 3 compares the physical activity level of patients with type 2 diabetes in different groups receiving omega-3 and Zn supplements. The ANOVA analysis model (by regulating the effect of base values) showed no significant difference between the four groups in terms of the average change in physical activity ($P=0.546$).

Table 4 compares the liver markers of patients with type 2 diabetes in different groups receiving omega-3 and Zn supplements. The ANOVA analysis model (by regulating the effect of all confounding variables) showed that the mutual effect of omega-3 and Zn supplements was significant on the average SGPT change ($P < 0.001$). Also the decrease in SGPT in the omega-3 group (after intervention) was associated with a remarkable reduction ($P=0.083$). The ANOVA analysis model (by regulating the effect of all confounding variables) showed that the effect of taking Zn supplement was significant on the average SGOT change ($P=0.013$). So that SGOT values in Zn supplement groups decreased by an average of 11.049 units. On the other hand, SGOT was significantly decreased in each of the intervention groups with omega-3, Zn and omega-3 with Zn ($P<0.001$). The effect of taking Zn supplement was significant on the moderate GGT change ($P=0.032$). After regulating the effect of the confounding variables, the GGT values in the groups receiving Zn supplement decreased by an average of 4.267 units. After 2 months of intervention there was a significant decrease in GGT in Zn group ($P=0.004$). Additionally, the decrease in GGT in Zn and omega-3 group was significant ($P=0.045$). It should be noted that the ANOVA analysis test for other liver markers (ALP and bilirubin and albumin) did not detect any significant difference ($P>0.05$).

Table 5 compares the Kidney markers of patients with type 2 diabetes in four experimental groups. The ANOVA analysis model (by regulating the effect of all confounding variables) showed that the effect of taking Zn supplement on the average uric acid level change was not significant, ($P=0.064$). Having regulated the effects of confounding variables, uric acid levels in Zn supplement groups decreased by an average of 0.744, while in other groups an average decrease of 0.211 units was observed. Furthermore, the decrease in uric acid in Zn group was significant after the intervention ($P<0.001$), while it was not significant in the omega- 3 group ($P=0.436$). After two months of intervention, the blood creatinine levels in the omega-3 group and omega-3 group with Zn decreased which was not significant, and showed no significant increase in the Zn group. Additionally, blood urea levels showed a no significant decrease after 2 months in Zn and omega-3 group.

Discussion:

This study was a randomized, controlled clinical trial which aimed to evaluate the effects of omega-3 and Zn supplements on liver markers (ALT, SGOT, SGPT, GGT, bilirubin and albumin) and Kidney markers (creatinine, uric acid and urea blood) in patients with type 2 diabetes. The liver is a complex and large organ whose main role is to design and manage the metabolism of carbohydrate, protein and fat. Alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) enzymes are present in the liver in normal amounts. An increase in the activity of these enzymes reflects liver dysfunction, resulting in leakage of liver cells into the blood

stream (Madani et al., 2009). There is an association between the increase in serum levels of liver markers and type 2 diabetes, although this association has been reported in many longitudinal studies. However, the results of these observations were different (André et al., 2005; Perry et al., 1998). The prospective studies have also shown a significant association between gamma glutamyl transferase (GGT) and the progression of certain diseases, including coronary heart disease and stroke. As a result, an increase in the gamma glutamyl transferase enzyme levels contributes to the prediction of progression of metabolic syndrome and type 2 diabetes (Nakanishi et al., 2004). ALP can be found in the cell membrane. As a result, it increases to a lesser extent in liver damages in the serum. However, AST and ALT iso-enzymes are present in the cytoplasm. So in the early stages of liver damages, an increase in the level of these enzymes is observed (Ajayi & Odutuga, 2004)]. Omega-3 fatty acids can play a key role in controlling the pathways of liver metabolism, given the role they play in the pathways of the peroxisome proliferator activated receptors (PPAR) and the inhibition of liver lipogenesis through expression of regulatory proteins (Jump, 2008; Xu et al., 1999). In the present study, SGOT blood level (AST) significantly decreased by consuming omega-3 fatty acids ($P<0.001$). The blood level of ALT (SGPT) also decreased by consuming omega-3 fatty acids ($P=0.193$) which was similar to a study by Dasarathy (Dasarathy et al., 2015) . In a study by (Spadaro et al., 2008) , the blood level of ALT improved after receiving omega-3 supplement. Studies have shown that the use of omega-3 fatty acids can improve the function and level of liver enzymes in liver patients, which may have anti-inflammatory and antioxidant effects due to the role that omega-3 fatty acids play in inhibiting triglyceride synthesis (Capanni et al., 2006; Mori & Beilin, 2004; Sarbolouki et al., 2010). Parker et al. also showed that the use of omega-3 fatty acids can have beneficial effects on liver markers and improve the blood levels of these enzymes (Parker et al., 2012). In summary, our findings are in good agreement with the numerous results of previous studies on the effects of omega-3 fatty acids on liver metabolism. Zn is an essential element for the metabolism of protein, carbohydrates and physiological functions. Many liver diseases and type 2 diabetes are associated with Zn deficiency in the body. Zn supplement can improve the neurological symptoms of liver encephalopathy and cirrhosis (Grüngreiff & Reinhold, 2005). Zn supplement can improve liver function because of its role in decreasing endotoxin, decreasing the production of inflammatory cytokine protein, decreasing oxidative stress and decreasing the death of hepatocyte cells (Mohammad et al., 2012). Zn can also prevent lipid peroxidation in the liver and thereby improve liver function with its effect on antioxidant potency (Sullivan et al., 1980). In this study, the SGOT blood level decreased significantly after Zn consumption ($P<0.001$). Additionally, the SGPT blood level also decreased after Zn consumption ($P=0.065$). The highest decrease in SGPT was observed in the Zn supplement group (4.528 units), which is consistent with several studies, shows improvement in liver function after Zn consumption. However, in a study by Parham, no significant changes were observed in levels of liver enzymes after Zn supplement (Parham, 2008). One of the side effects of diabetes is long-term chronic Kidney disease (Burrows et al., 2008). Several factors contribute to the progression of diabetic and non-diabetic glomerulopathy, such as systemic and glomerular hypertension, albuminuria, dietary intake, and hyperlipidemia, In this study, the amount of creatinine, urea and uric acid were evaluated in order to investigate the Kidney function. According to Miller, omega-3 fatty acid supplements was able to improve the renal function in patients with diabetes by decreasing the protein excretion (Miller et al., 2013). In another meta-analysis, omega-3 fatty acid supplements were able to improve the renal function by decreasing the urinary protein excretion (Miller et al., 2009). Omega-3 fatty acids, which have an effect on vascular function and hemodynamic kidney function, can play a role in improving the liver function (Stirban et al., 2010; Shapiro et al., 2011). By examining the effects of omega-3 supplements on blood pressure, the previous meta-analyses showed that the addition or a relatively high dose of omega-3 fatty acids caused a significant decrease in blood pressure. Low blood pressure may decrease renal perfusion and lower the GFR, decrease the excretion of protein and improve the hemodynamic effects of the kidney (Clark et al., 1993). The results of this study are based on kidney function, independent of protein excretion, glomerular filtration or GFR. Omega-3 fatty acids which affect the production of prostaglandins and boost immune system and vasodilation, can reduce the progression of kidney disease in patients with diabetes. These fatty acids affect the erythrocyte membrane and decrease the renal ischemia(Stirban et al., 2010; Di et al., 2004; Massaro et al., 2008). In another study, omega-3 fatty acids did not have an effect on the improvement of kidney function or the progression of kidney disease in patients with diabetes (Rossing et al., 1996). Possible reasons for this inconsistency are attributed to the difference in the pathology of kidney disease or the prevalence of risk factors for disease progression, the difference in outcome evaluation, sample size, and the limitation of the quality of the study. The oxidative stress plays many roles in the pathogenesis of nephropathy in diabetes. Oxidative stress can be caused by increased production of reactive oxygen species (ROS) or antioxidant deficiency, Accordingly, Zn can be one of the effective factors in strengthening the antioxidant system (Kelly, 1998). Given these considerations, it seems logical that antioxidants, such as Zn, may be used to treat or prevent diabetic nephropathy. In a study, Zn decreased the protein excretion from urine (Kadhim et al., 2006). Another risk factor for nephropathy is hyperlipidemia which has been shown to have beneficial effects on blood lipid levels (El-Ashmony et al., 2012). In the present study, the level of uric acid was significantly decreased with Zn after intervention. This indicates improvement in renal function with regard to uric acid index in these patients, which is similar to the study conducted by (El-Ashmony et al., 2012). According to Parham (Parham, 2008) , the amount of protein released from the renal also decreased after taking Zn. Khadim (Kadhim et al., 2006) found that serum creatinine did not significantly change after taking the supplement. His study did not show a significant effect on serum urea and creatinine. This could be attributed to the normal Zn blood levels in this study, unlike other studies. Creatinine levels decreased in omega-3 and Zn groups after intervention which could be attributed to the role of omega-3 in decreasing creatinine levels in the blood. In this study, there was no significant effect on kidney markers after omega-3 consumption.

Conclusion:

Since the use of omega-3 and Zn in Iran is low and these nutrients are lower in patients with diabetes than in the general population, it seems that taking these dietary supplements can improve their lives. In sum, the omega-3 and Zn supplements, alone or simultaneously, can improve the relative performance of the liver and kidneys in patients with diabetes. On the other hand, the need to do further studies with other doses of these supplements in this regard is necessary.

References:

Ajayi OB, Odutuga A (2004) Effect of low-zinc status and essential fatty acids deficiency on the activities of aspartate aminotransferase and alanine aminotransferase in liver and serum of albino rats. Molecular Nutrition & Food Research 48 (2):88-90.

Alberti KGMM, Zimmet Pf (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabetic medicine 15 (7):539-553.

André P, Balkau B, Born C, Royer B, Wilpart E, Charles M-A, Eschwège E (2005) Hepatic markers and development of type 2 diabetes in middle aged men and women: a three-year follow-up study: the DESIR study (Data from an Epidemiological Study on the Insulin Resistance syndrome). Diabetes & metabolism 31 (6):542- 550.

Burrows NR, Li Y, Williams DE (2008) Racial and ethnic differences in trends of end-stage renal disease: United States, 1995 to 2005. Advances in chronic kidney disease 15 (2):147-152.

Capanni M, Calella F, Biagini M, Genise S, Raimondi L, Bedogni G, Svegliati-Baroni G, Sofi F, Milani S, Abbate R (2006) Prolonged n-3 polyunsaturated fatty acid supplementation ameliorates hepatic steatosis in patients with non-alcoholic fatty liver disease: a pilot study. Alimentary pharmacology & therapeutics 23 (8):1143-1151.

Clark WF, Parbtani A, Naylor CD, Levinton CM, Muirhead N, Spanner E, Huff MW, Philbrick DJ, Holub BJ (1993) Fish oil in lupus nephritis: clinical findings and methodological implications. Kidney international 44 (1):75-86.

Coleman JE (1992) Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. Annual review of biochemistry 61 (1):897-946.

Coste TC, Gerbi A, Vague P, Pieroni G, Raccah D (2003) Neuroprotective effect of docosahexaenoic acid- enriched phospholipids in experimental diabetic neuropathy. Diabetes 52 (10):2578-2585.

Dasarathy S, Dasarathy J, Khiyami A, Yerian L, Hawkins C, Sargent R, McCullough AJ (2015) Double blind randomized placebo controlled clinical trial of omega 3 fatty acids for the treatment of diabetic patients with nonalcoholic steatohepatitis. Journal of clinical gastroenterology 49 (2):137.

Dasarathy S, Dasarathy J, Khiyami A, Yerian L, Hawkins C, Sargent R, McCullough AJ (2015) Double- blind randomized placebo-controlled clinical trial of omega 3 fatty acids for the treatment of diabetic patients with nonalcoholic steatohepatitis. Journal of clinical gastroenterology 49 (2):137-144.

Dashti HM, Mathew TC, Jadaon MM, Ashkanani E (1997) Zinc and liver cirrhosis: biochemical and histopathologic assessment. Nutrition 13 (3):vi-212.

Di Stasi D, Bernasconi R, Marchioli R, Marfisi RM, Rossi G, Tognoni G, Tacconi MT (2004) Early modifications of fatty acid composition in plasma phospholipids, platelets and mononucleates of healthy volunteers after low doses of n-3 polyunsaturated fatty acids. European journal of clinical pharmacology 60 (3):183-190.

Ducheix S, Montagner A, Polizzi A, Lasserre F, Marmugi A, Bertrand-Michel J, Podechard N, Al Saati T, Chétiveaux M, Baron S (2013) Essential fatty acids deficiency promotes lipogenic gene expression and hepatic steatosis through the liver X receptor. Journal of hepatology 58 (5):984-992.

El-Ashmony SMA, Morsi HK, Abdelhafez AM (2012) Effect of Zinc Supplementation on Glycemic Control, Lipid Profile, and Renal Functions in Patients with Type II Diabetes: A Single Blinded, Randomized, Placebo-Controlled, Trial. Journal of Biology, Agriculture and Healtcare 2 (6):33-37.

Fraser A, Harris R, Sattar N, Ebrahim S, Smith GD, Lawlor DA (2009) Alanine aminotransferase, γ- glutamyltransferase, and incident diabetes The British Women's Heart and Health Study and meta-analysis. Diabetes care 32 (4):741-750.

Grüngreiff K, Reinhold D (2005) Liver cirrhosis and “liver” diabetes mellitus are linked by zinc deficiency. Medical hypotheses 64 (2):316-317.

Han E, Yun Y, Kim G, Lee Y-h, Wang HJ, Lee B-W, Cha BS, Kim BS, Kang ES (2016) Effects of Omega- 3 Fatty Acid Supplementation on Diabetic Nephropathy Progression in Patients with Diabetes and Hypertriglyceridemia. PloS one 11 (5):e0154683.

Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR (2012) Management of hyperglycaemia in type 2 diabetes: a patient-centered approach. Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia 55 (6):1577-1596.

Jacobson H (1991) Chronic renal failure: pathophysiology. The Lancet 338 (8764):419-423.

Jump DB (2008) N-3 polyunsaturated fatty acid regulation of hepatic gene transcription. Current opinion in lipidology 19 (3):242.

Kadhim HM, Ismail SH, Hussein KI, Bakir IH, Sahib AS, Khalaf BH, Hussain SAR (2006) Effects of melatonin and zinc on lipid profile and renal function in type 2 diabetic patients poorly controlled with metformin. Journal of pineal research 41 (2):189-193.

Kakkar R, Mantha SV, Radhi J, Prasad K, Kalra J (1998) Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. Clinical Science 94 (6):623-632.

Kelly F (1998) Use of antioxidants in the prevention and treatment of disease. Journal of the International Federation of Clinical Chemistry 10 (1):21-23.

Kim HJ, Lee KT, Park YB, Jeon SM, Choi MS (2008) Dietary docosahexaenoic acid-rich diacylglycerols ameliorate hepatic steatosis and alter hepatic gene expressions in C57BL/6J-Lepob/ob mice. Molecular nutrition & food research 52 (8):965-973.

Kromhout D, Yasuda S, Geleijnse JM, Shimokawa H (2011) Fish oil and omega-3 fatty acids in cardiovascular disease: do they really work? European heart journal 33 (4):436-443.

Lehto S, Niskanen L, Rönnemaa T, Laakso M (1998) Serum uric acid is a strong predictor of stroke in patients with non–insulin-dependent diabetes mellitus. Stroke 29 (3):635-639.

Madani H, Rahimi P, Mahzouni P (2009) Effects of hydroalcoholic extract of Juglans regia leaves on activity of AST and ALT enzymes in alloxan-induced diabetic rats.

Maes M, Christophe A, Delanghe J, Altamura C, Neels H, Meltzer HY (1999) Lowered ω3 polyunsaturated fatty acids in serum phospholipids and cholesteryl esters of depressed patients. Psychiatry research 85 (3):275- 291

Mahmoud Parham MA (2008) Effect of Zinc Supplementation on Microalbuminuria in Patients With Type 2 Diabetes: A Double Blind, Randomized, Placebo-Controlled, Cross-Over Trial the review of diabetic studies 5 (2):102-109

Maret W, Sandstead HH (2006) Zinc requirements and the risks and benefits of zinc supplementation. Journal of Trace Elements in Medicine and Biology 20 (1):3-18

Massaro M, Scoditti E, Carluccio MA, Montinari MR, De Caterina R (2008) Omega–3 Fatty Acids, Inflammation and Angiogenesis: Nutrigenomic Effects as an Explanation for Anti-Atherogenic and Anti- Inflammatory Effects of Fish and Fish Oils. Journal of nutrigenetics and nutrigenomics 1 (1-2):4-23

Miller ER, Juraschek SP, Anderson CA, Guallar E, Henoch-Ryugo K, Charleston J, Turban S, Bennett MR, Appel LJ (2013) The Effects of n-3 Long-Chain Polyunsaturated Fatty Acid Supplementation on Biomarkers of Kidney Injury in Adults With Diabetes Results of the GO-FISH trial. Diabetes care 36 (6):1462-1469

Miller ER, Juraschek SP, Appel LJ, Madala M, Anderson CAM, Bleys J, Guallar E (2009) The effect of n–3 long-chain polyunsaturated fatty acid supplementation on urine protein excretion and kidney function: meta- analysis of clinical trials. Am J Clin Nutr 89 (6):1937-1945. doi:10.3945/ajcn.2008.26867

Modan M, Halkin H, Karasik A, Lusky A (1987) Elevated serum uric acid—a facet of hyperinsulinaemia. Diabetologia 30 (9):713-718

Mohammad MK, Zhou Z, Cave M, Barve A, McClain CJ (2012) Zinc and liver disease. Nutrition in Clinical Practice 27 (1):8-20

Mori TA, Beilin LJ (2004) Omega-3 fatty acids and inflammation. Current atherosclerosis reports 6 (6):461- 467

Nakanishi N, Suzuki K, Tatara K (2004) Serum γ-glutamyltransferase and risk of metabolic syndrome and type 2 diabetes in middle-aged Japanese men. Diabetes care 27 (6):1427-1432

Özcelik D, Nazıroglu M, Tunçdemir M, Çelik Ö, Öztürk M, Flores-Arce M (2012) Zinc supplementation attenuates metallothionein and oxidative stress changes in kidney of streptozotocin-induced diabetic rats. Biological trace element research 150 (1-3):342-349

Parker HM, Johnson NA, Burdon CA, Cohn JS, O'Connor HT, George J (2012) Omega-3 supplementation and non-alcoholic fatty liver disease: a systematic review and meta-analysis. Journal of hepatology 56 (4):944- 951

Perry IJ, Wannamethee SG, Shaper AG (1998) Prospective study of serum γ-glutamyltransferase and risk of NIDDM. Diabetes Care 21 (5):732-737

Rossing P, Hansen BV, Nielsen FS, Myrup B, Hølmer G, Parving H-H (1996) Fish oil in diabetic nephropathy. Diabetes Care 19 (11):1214-1219

Sarbolouki S, Djalali M, Dorosty A, Djazayery S, Eshraghian M, Ebadi S, Hashemi S (2010) Effects of EPA and vitamin E on serum enzymatic antioxidants and peroxidation indices in patients with type II Diabetes Mellitus. Iranian journal of public health 39 (3):82

Shapiro H, Theilla M, Attal-Singer J, Singer P (2011) Effects of polyunsaturated fatty acid consumption in diabetic nephropathy. Nature Reviews Nephrology 7 (2):110

Spadaro L, Magliocco O, Spampinato D, Piro S, Oliveri C, Alagona C, Papa G, Rabuazzo A, Purrello F (2008) Effects of n-3 polyunsaturated fatty acids in subjects with nonalcoholic fatty liver disease. Digestive and Liver Disease 40 (3):194-199

Stirban A, Nandrean S, Götting C, Tamler R, Pop A, Negrean M, Gawlowski T, Stratmann B, Tschoepe D (2010) Effects of n–3 fatty acids on macro-and microvascular function in subjects with type 2 diabetes mellitus– . The American journal of clinical nutrition 91 (3):808-813

Sullivan J, Jetton M, Hahn H, Burch R (1980) Enhanced lipid peroxidation in liver microsomes of zinc- deficient rats. The American journal of clinical nutrition 33 (1):51-56

Sun Q, Van Dam RM, Willett WC, Hu FB (2009) Prospective study of zinc intake and risk of type 2 diabetes in women. Diabetes care 32 (4):629-634

Tomat AL, de los Ángeles Costa M, Arranz CT (2011) Zinc restriction during different periods of life: influence in renal and cardiovascular diseases. Nutrition 27 (4):392-398

WHO (2016) Global Report on Diabetes. Geneva

Xu J, Nakamura MT, Cho HP, Clarke SD (1999) Sterol Regulatory Element Binding Protein-1 Expression Is Suppressed by Dietary Polyunsaturated Fatty Acids A Mechanism For The Coordinate Suppression of Lipogenic Genes by Polyunsaturated Fats. Journal of Biological Chemistry 274 (33):23577-23583.

Table 1. Comparison of the demographic and background variables of the patients with diabetes type 2 in the groups receiving zinc and omega 3 supplementation

Variable	Category	Experimental group				P-value *
		Omega-3	Zinc	Omega-3 and zinc	Control	
Age (year)	-	58.0 **(13.0)	(8.0) 57.0	(7.0) 53.0	(8.0) 54.5	0.089
Years of affliction with the disease	-	(5.0) 5.0	(2.0) 4.0	(3.0) 4.0	(2.0) 4.5	0.553
BMI	-	(34.34) 4.56	(31.57) 3.88	(30.25) 4.34	(31.89) 4.27	0.319
Gender	Female	**(52.2%) 12	(57.1%) 12	(57.1%) 12	(61.1%) 11	0.953
	Male	(47.8%) 11	(42.9%) 9	(42.9%) 9	(38.9%) 7	
Educational level	Illiterate	(8.7%) 2	(23.8%) 5	(33.3%) 7	(50.0%) 9	0.126
	Elementary or junior school	(39.1%) 9	(38.1%) 8	(23.8%) 5	(16.7%) 3	
	High school and above	(52.2%) 12	(38.1%) 8	(42.9%) 9	(33.3%) 6	
Job	Housekeeper	(52.2%) 12	(57.1%) 12	(57.1%) 12	(50.0%) 9	0.147
	Retired	(39.1%) 9	(9.5%) 2	(28.6%) 6	(16.7%) 3	
	Other jobs	(8.7%) 2	(33.3%) 7	(14.3%) 3	(33.3%) 6	
Place of living	City	(95.7%) 22	(100.0%) 21	(95.2%) 20	(94.4%) 17	0.778
	Village	(4.3%) 1	(0.0%) 0	(4.8%) 1	(5.6%) 1	

*Kruskal–Wallis test was used to compare quantitative variables in the groups and chi-square test was used to compare qualitative variables.

**Median (interquartile range) was used to describe quantitative variables and frequency (percentage) was used to describe qualitative variables.

Table 2. Comparison of the received amount of nutrition by the patients with diabetes type 2 in the groups receiving zinc and omega 3 supplementation

Variable	Experimental group												P-value*
	Omega3 (n=23)			Zinc (n=21)			Omega3 and zinc (n=21)			Control (n=18)			
	Before	after	Change	before	after	change	before	after	change	before	after	change	
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	
Energy Kcal	1912.229±21.3	1765.178±78.53	-146.235±43.9	1990.427±85.6	1890.286±66.9	-100.365±19.6	1882.178±52.6	1841.201±95.2	-40.239±57.5	1951.223±27.6	1766.151±11.4	-185.237±16.1	0.156
Protein g/d	68.13±36.9	69.11±33.2	0.10±97.3	68.11±11.5	67.12±55.6	0.1±56.87	69.11±08.1	71.10±36.9	2.11±28.1	66.10±60.7	70.9±14.4	3.8±53.7	0.638
CHO g/d	236.40±37.7	231.43±42.4	-4.43±94.6	228.33±86.9	238.40±58.8	9.34±72.7	240.47±490.7	244.37±43.5	3.40±93.6	237.29±59.5	227.17±93.3	9.31±66.5	0.351
Fat g/d	74.14±77.7	67.12±89.2	-6.15±88.8	72.16±54.9	66.9±46.8	6.18±07.1	72.15±67.4	63.10±83.1	-8.14±84.2	74.12±30.8	71.13±36.2	-2.15±93.6	0.248

SFA g/d	PUFA g/d	MUFA g/d	Glucose g/d	Cholesterol Mg/d	Dietary fiber g/d	Insoluble fiber g/d	Zinc Mg/d
17.3±72.6	21.6±10.8	/259±62.6	63/21±48/1	237.136±44.6	14/3±55/3	3.1±46.6	10.2±33.9
17.3±51.8	29.10±16.8	25.8±72.8	61/15±77/2	258.131±54.3	14/3±36/4	3.1±15.1	10.2±40.9
-0.5±20.2	8.10±05.7	0.7±1.2	21/3±1/71-	21.69±09.2	2/7±0/19-	-0.1±31.7	0.1±06.2
16.3±85.1	23.15±74.5	21/5±82/6	18/2±60/95	211.82±99.2	5/2±17/10	6.3±39.7	10.3±67.7
17.3±61.8	22.13±35.2	21.5±31.6	17/5±63/84	217.57±34.7	5/0±17/69	2.1±88.2	11.4±00.5
0.4±76.4	1.5±38.3	-0.3±51.1	21/3±2/89	5.76±34.8	6/4±0/59	-3.4±50.2	0.3±33.3
18.3±38.3	23.8±92.4	21.6±71	17/7±52/19	237.96±83.8	3/9±15/11	2.1±40.3	11.4±54.4
17.3±04.6	20.4±95.3	19.5±77.2	16/7±59/49	227.78±82.6	3/1±15/73	2.0±13.6	11.4±41.5
-1.5±34.9	-2.6±97.3	-1.4±93.9	9/9±7/30	-1.87±0.4	4/1±0/62	0.1±27.4	-0.6±13.4
14.3±67.1	29.13±24.3	21.6±16.6	14/8±52/61	233.111±78.2	3/9±14/18	2.0±46.9	10.2±23.9
14.2±09.6	25.9±48.1	18.4±30.7	17/2±55/40	252.109±05.4	2/7±13/52	2.0±44.9	10.3±39.0
-0.4±57.0	-3.8±76.8	-2.7±86.4	7/3±2/79	18.89±26.3	2/7±0/65-	-0.1±03.3	0.3±16.3
0.010	<0.001	0.019	0/676	0.405	0/035	0.008	0.955

*The ANCOVA analysis model with modification of the effects of the basic values was used for comparing the four groups.

Table 3. Comparison of the rate of physical activity in the patients with diabetes type 2 in the groups receiving omega3 and zinc supplementation(MET.h/day)

Omega-3 (n=23)				Zinc (n=21)				Omega-3 and Zinc (n=21)				Control (n=18)				P-value**
before	after	Change		Before	After	change		before	after	change		before	after	change		
Mean ±SD	Mean ±SD	Mean ±SD	P-value*	Mean ±SD	Mean ±SD	Mean ±SD	P-value*	Mean ±SD	Mean ±SD	Mean ±SD	P-value*	Mean ±SD	Mean ±SD	Mean ±SD	P-value*	
41.1±16.6	43.0±17.7	1.9±6.3	0.581	39.8±18.5	42.2±20	2.4±5.5	0.607	38.3±2.14	39.2±19.5	0.9±5.3	0.866	40.0±15.7	43.6±17.8	2.1±6.7	0.394	0.546

* shows the comparison the before and after values in each group.

** shows the comparison between the four groups in terms of the average value change.

Table 4. Comparison of the liver markers of the patients with type 2 diabetes in groups receiving omega-3 and Zn supplement

Variable	Omega-3 (n =23)				Zn (n =21)				Omega-3 and Zn (n =21)				Control (n=18)				(By regulating the effect of confounding variables)		
	Before	After	Change		Before	After	Change		Before	After	Change		Before	After	Change				
	Mean±SD	Mean±SD	Mean±SD	P-value*	Mean±SD	Mean±SD	Mean±SD	P-value*	Mean±SD	Mean±SD	Mean±SD	P-value*	Mean±SD	Mean±SD	Mean±SD	P-value*	P-value **	P-value +	P-value ++
(U/L)SGOT	11/9±33/21	8/5±24/26	9/2±8/95-	<0/001	11/1±31/38	6/6±19/80	9/0±11/57-	<0/001	7/4±28/76	6/3±19/85	7/1±8/90-	<0/001	24/05±4/2	8/1±22/05	-2±7.1	0.251	0/466	0/013	0/653
(U/L) SGPT	18/1±32/39	14/9±28/34	14/4±4/04-	0/193	14/8±23/90	12/7±21/19	6/3±2/71-	0/065	9/7±22/61	9±19/57	7/6±3/04-	0/083	10/2±24/72	10/6±24/72	6/5±0/00	>0/999	0/028	0/602	<0/001
(U/L)ALP	72/8±181/65	67/6±193/39	59/5±11/73	0/355	47/9±182/52	60±203/38	30/6±20/85	0/005	63/3±188/47	49/2±190/80	49/5±2/33	0/831	82/9±186/38	74/5±203/55	63±17/16	0/264	0/371	0/864	0/978
(UI/L) GGT	10/7±25/74	8/8±24/62	9/3±1/11-	0/573	14/5±31/69	10/9±26/33	7/6±5/35-	0/004	9/8±23/09	9/7±21/16	4/1±1/93-	0/045	11/9±27/15	10/5±27/07	2/9±0/08-	0/906	0/929	0/032	0/566
g/dl Albumin	0/5±4/60	0/28±4/43	0/45±0/16-	0/094	0/5±4/44	0/2±4/55	0/5±0/11	0/373	0/3±4/49	0/3±4/44	0/3±0/04-	0/535	0/2±4/51	0/2±4/51	0/1±0/00	0/884	0/104	0/372	0/809
mg/dl Total Bilirubin	0/1±0/54	0/2±0/62	0/1±0/07	0/023	0/3±0/65	0/1±0/57	0/4±0/07-	0/459	0/1±0/56	0/4±0/68	0/3±0/12	0/177	0/1±0/57	0/1±0/52	0/1±0/05-	0/050	0/117	0/532	0/518
mg/dl Bilirubin	0/13±0/16	0/06±0/17	0/1±0/01	0/629	0/06±0/14	/04±0/15	0/04±0/01	0/160	0/08±0/14	0/09±0/18	0/12±0/03	0/215	2/5±0/79	/15 0/08±0	2/5±0/63-	0/310	0/370	0/834	0/615

* shows the significance of the before and after comparison of the dependent variables in each experimental group using Wilcoxon test.
**shows the significance of the major effect of omega-3 supplementation on the average change of responses (it compares the groups receiving omega-3 supplementation with other groups).
+ shows the significance of the major effect of zinc supplementation on the average change of responses (it compares the groups receiving zinc supplementation with other groups).
++ shows the significance of the mutual effect of omega-3 and zinc supplementation on the average change of responses.

Table 5. Comparison of the Kidney markers of the patients with type 2 diabetes in groups receiving omega-3 and Zn supplement

Variabl	**Omega-3(n=23)**			**Zn(n=21)**			**Omega-3 and (n=21) Zn**			**Control (n=18)**			**(By regulating the effect of confounding variables)**
	Before	**After**	**Change**	**Before**	**After**	**Change**	**Before**	**After**	**Change**	**Before**	**After**	**Change**	

	Mean±SD	**Mean±SD**	**Mean±SD**	**P-value***	**Mean±SD**	**Mean±SD**	**Mean±SD**	**P-value***	**Mean±SD**	**Mean±SD**	**Mean±SD**	**P-value***	**Mean±SD**	**Mean±SD**	**Mean±SD**	**P-value***	**P-value ****	**P-value +**	**P-value ++**
mg/dl Creatinine	0/1±1/07	0/1±1/0	0/1±0/002-	0/952	0/1±1/02	0/1±1/03	0/05±0/009	0/430	0/2±1/03	0/2±1/01	0/1±0/02-	0/341	0/1±0/93	0/1±0/94	0/07±0/01	0/479	0/309	0/997	0/943
mg/dl Urea	7/4±34/65	9/7±33/86	12±0/78-	0/758	6/7±33/09	8±31/23	7±1/85-	0/240	11/9±33/95	11/1±31/42	6/8±2/52-	0/108	28/16±5/3	9/9±28/61	- 0/44±8/3	0/824	0/984	0/599	0/969
mg/dl Acid Uric	1/1±5/44	1/3±5/23	1/2±0/20-	0/436	0/9±5/60	0/7±4/68	0/8±0/91-	<0/001	1/8±5/95	1/6±5/32	1/6±0/63-	0/097	0/9±5/23	1±5/13	0/0/10±8-	0/613	0/365	0/064	0/242

* shows the significance of the before and after comparison of the dependent variables in each experimental group using Wilcoxon test.

**shows the significance of the major effect of omega-3 supplementation on the average change of responses (it compares the groups receiving omega-3 supplementation with other groups).

+ shows the significance of the major effect of zinc supplementation on the average change of responses (it compares the groups receiving zinc supplementation with other groups).

++ shows the significance of the mutual effect of omega-3 and zinc supplementation on the average change of responses.